Chapter 57

Nematodes as Indicators of Toxic Environmental Contaminants

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Environments are not contaminated by a single chemical species; they are exposed to a range of potentially toxic materials from natural and human sources. Every contaminant in the system can produce a series of compounds resulting from photolysis, biological conversion, or chemical interaction among contaminants or natural components. Biological effects produced by the total mix of contaminants can result from synergistic or antagonistic action of the various constituents of the ecosystem.

The toxic effects of contaminants fall into several classes: 1) immediate risk to survival, 2) irreversible damage to some physiological process, 3) reversible damage to some physiological process, or 4) long-term, low-frequency risk to individuals (teratogenesis, carcinogenesis, mutagenesis).

The range of biological effects produced by single or mixed contaminants is further compounded by significant differences in sensitivity to the contaminants by different species in the environment, and by significant differences in sensitivity among members of a single population. These differences are due to many factors, including differences in patterns of uptake and differences in ability to detoxify contaminants.

The major problem in environmental toxicology is the detection of conditions that could result in some adverse effect on natural populations in light of the complexities outlined above. Most monitoring programs focus on the presence and concentrations of individual chemicals rather than on the net toxic effects in a particular environment. Usually such monitoring is carried out by chemical analysis, looking for individual compounds from a toxic chemicals list, and determining whether these chemicals individually occur at concentrations greater than some previously established "safe level." Another approach that is becoming more widely used is biomonitoring—the application of one or more biological systems as indicators of the quality of contaminated environments. Biomonitoring is an approach that focuses on the combined effects of environmental contaminants. It can be either field based, using the actual response of organisms in situ, or laboratory based, using appropriate indicator organisms. This review will examine the use of nematodes in biomonitoring.

Free-living nematodes have several features that make them excellent indicator organisms for determination of the presence of toxic contaminants in aquatic, marine, and terrestrial environments. Among these features are a life cycle that is highly entrained to the environment in which they are tested and a nearly ubiquitous distribution in nature. Nematodes can be used as field indicator species or as laboratory "yardstick" test organisms. Nematodes are extremely hardy field organisms; for the majority of chemicals, the lethal concentration required is much greater for nematodes than for most other field organisms. However, the pattern of growth of either natural or laboratory populations of nematodes is a good indicator of the presence of toxic materials (22,23,26,27), providing an effective yardstick for physiological or gene-level damage.

The broad base of experimental and descriptive studies on the life-cycle and genetics of this groups, makes nematodes an ideal group to be used for bioassays. Since the publication of Brenner's paper on the genetics of *Caenorhabditis elegans* (9), free-living nematodes have become widely used organisms in developmental biology, neurobiology, and genetic research, greatly adding to the understanding of the basic biology of this group of organisms.

Field Ecotoxicological Studies

In most ecosystems there is a wide diversity of nematode species, due in part to the wide range of trophic levels they occupy (27). Free-living nematodes may be consumers of bacteria, grazers of primary producers, or predators. Nematode populations are easy to locate and are present in large numbers suitable for statistical analysis. However, the identification of the individual species present usually requires expertise in nematode systematics. Many physical properties of an ecosystem will affect the diversity of nematodes (31). Sediment or soil characteristics affect the population density and species diversity (14). Seasonal fluctuations cause changes in population density and diversity of species (14). There is also a significant influence of temperature on species composition even in relatively stable environments (31).

The usual response of field nematodes to toxic contaminants is an increase in population numbers of tolerant species (2). In studies of aquatic ecosystems Callahan et al. (10) found that the total numbers of nematodes present were significantly correlated to nitrite-nitrogen levels and the number of species at the sites was correlated with medium particle size. Bisessar (6) found a negative relationship between the soil concentration of heavy metals and population levels of terrestrial nematodes. Howell (15,16) reported greater heavy metal concentrations and reductions in populations levels in nematodes from polluted sites than from an unpolluted site. Leethan et al. (19) reported a significant reduction in population numbers with addition of SO_2 when compared with the control. Kathman et al. (17) found that a 1:3 mixture of wood waste to sediment produced optimal conditions for benthic nematode communities.

Raffaelli and Mason (21) proposed the use of the nematode to copepod ratio for biomonitoring sewage pollution. There is a negative correlation between the nematode:copepod ratio and the median particle size. Ratios from clean sampling stations were always less than 100, whereas those from polluted sampling stations were always more than 100. Warwick (32) proposed adjusting the nematode:copepod ratio to compensate for sediment granulometry. The nematode:copecod ratio is approximately 40 for polluted fine sediments and approximately 10 for polluted sands. The ratio works well for oil spills, as copepods are more sensitive to oil pollution than nematodes. Raffaelli and Mason (21) state the limiting factor of such meiofauna population analysis is that the populations must respond to pollution before it becomes obvious visually.

Coull et al. (11) contest the validity of the use of nematode:copepod ratio as a biomonitoring tool since errors can be introduced by natural fluctuations in population size. Amjad and Gray (5) used the nematode:copepod ratio along a known gradient of organic pollution and found that copepod numbers decreased and nematode numbers increased along the gradient of increasing organic enrichment.

The utilization of nematodes as field indicators has not yet been widely applied, but any relative increase in the relative abundance of nematodes in an ecosystem should be considered an early warning of toxic contamination.

Laboratory Bioassay Organisms

Panagrellus redivivus, which has wide applicability in a biomonitoring system, has been used to detect the toxicity of complex environmental samples and chemical fractions of such samples (20,22–30). The nematode is dioecious and ovoviviparous, and the first juvenile stage (J1) occurs in utero. Development from the first free-swimming stage (J2) through the adult takes 96 hours under standard culture conditions. During this developmental period the animal grows through three juvenile stages (J2, J3, J4). Each stage is separated from the previous stage by a molt, during which a new cuticle is formed and the old cuticle shed. The four postembryonic stages are each characterized by a specific size range: J2 = 250–350 μm, J3 = 350–550 μm, J4 = 550–750 μm, and adult = 750–2,000 μm (23). Growth from one stage to the next requires normal functioning of physiological and informational processes; growth will be inhibited at or before a molt in response to toxic materials that inhibit these processes. The *P. redivivus* test examines the growth and survival of a population of animals initially

arrested at the J2-J3 molt. The test exploits the fact that J2 *P. redivivus* require nutrients to grow beyond the J2-J3 molt. Stock cultures are maintained on a substrate of 1.7% agar supplemented with cholesterol and periodically fed with several drops of 1% baker's yeast in M9 phosphate buffer (9). Gravid females are removed from stock plates, washed in M9 buffer, and placed in a 2-mm-deep layer of M9 buffer on 1.7% agar plates for a 24-hour period. In this nonnutrient medium, the progeny J2 grow and produce the J3 cuticle, but further development is arrested; the J2-J3 do not undergo ecdysis of the J2 cuticle. Each female produces approximately 25 such arrested juveniles in a 24-hour period. A population of 100 such arrested J2-J3 animals are grown in the presence of limited nutrient and the substances to be tested. An untreated control population of arrested animals in limited nutrient medium (23) is also set up. Each test and control population is set up as 10 replicates of 10 animals each in 0.5 ml of fluid. After a 96-hour growth period, approximately 50% of the control population has reached the adult stage. The number of surviving animals in each test and control population is counted, and the stage distribution (numbers of J2, J3, J4, and adult animals) in each population is determined.

Three parameters are calculated from the survival and stage distribution. These are 1) *survival*, the percentage of the initial test population that survives the growth period relative to the number of survivors in the control population; 2) *growth*, the proportion of the test population that reaches the J4 or adult stage during the 96-hour growth period relative to this value in the control population; and 3) *maturation*, the proportion of the J4 and adult animals that reach the adult stage, relative to this value in controls. Survival reflects the action of the tested material on those physiological processes essential for life; growth reflects the effect of tested material on nonessential processes; and inhibition of maturation indicates inhibition of the utilization of genetic information (by mutagenesis or by blockage of transcription or translation). During *P. redivivus* postembryonic devolopment, genetic information is primarily required at the time of the J4-adult molt (23,27); the sensitivity of *P. redivivus* to agents that interfere with the flow of genetic information is greatest at this molt.

The *Panagrellus* bioassay can, therefore, discriminate three types of toxic end point: *lethality*—where a significant proportion of the test population dies, relative to the number that die in the control population; *inhibition*—where a significant proportion of the test population fails to grow to the J4 or adult stages relative to the control population; *stimulation*—where significantly more of the test population reaches the J4 and adult stages than is observed in the control population; and *phenotoxicity*—where the tested material specifically inhibits the completion of the J4-adult molt. Each of these effects can be quantified and the combined effects can be expressed as a single value—"fitness" (27), a weighted mean of the survival, growth, and maturation of the test population (survival has a weighted value double that of growth, and growth has a weighted value double that of maturation, thus reflecting the relative ecotoxicological importance of these three parameters). Fitness can range from 100 (growth and survival of test animals equal to that of the controls) to 0 (no survival).

The *P. redivivus* bioassay has been used to determine the toxic effects of approximately 400 single chemicals. The chemicals may be in solution or in a gas phase over an agar surface. Table 1 shows the response of *P. redivivus* to 10^{-6} M solutions of 18 known or suspected carcinogens and 7 metal compounds. Four of these compounds (5-bromouracil, proflavine, mercuric chloride, and methyl mercury) were lethal, nine significantly ($P < 0.05$) inhibited growth, and fourteen had phenotoxic effects. Only benzene, chloroform, ethyl methane sulphonate, and 3-methylcholantrene chloroform did not show phenotoxicity.

While the *P. redivivus* bioassay can be used to establish the toxicity of individual compounds, its real value lies in its applicability as a rapid cost-effective bioassay for samples from contaminated environments. Table 2 shows the fitness and overall effect found in elutriates of sediments chronically contaminated by polychlorinated

TABLE 1. Fitness and effects of 96-hour exposure of *Panagrellus redivivus* to 10^{-6} M aqueous solution of various known toxic chemicals.

Compound	Survival	Growth	Mature	Fitness
Acrylonitrile	100 –	100 –	58 P	94 P
Aflatoxin B	93 –	68 I	6 P	73 I
Aminobiphenyl	95 –	97 –	64 P	91 P
Benzene	93 –	96 –	92 –	93 –
5-Bromouracil	87 L	76 I	41 P	77 L
Chloromethyl ethyl ether	100 –	60 I	76 P	85 I
Dimethyl benzanthracine	100 –	97 –	77 P	95 P
Ethyl methanesulphonate	90 –	105 –	122 –	98 –
3-methylcholanthrine	95 –	87 I	99 –	93 I
Methyl methanesulphonate	92 –	86 I	76 P	88 I
Proflavine	79 L	65 I	58 P	72 L
β-Propiolactone	100 –	97 –	76 P	95 P
Vinyl chloride (gas)	100 –	85 I	58 P	89 I
Chloroform	99 –	95 –	89 –	96 –
Dibutyl pthalate	99 –	91 –	52 P	90 P
Dichloromethane	100 –	86 I	15 P	83 I
Hexachlorobenzene	100 –	59 I	78 P	85 I
Methoxychlor	93 –	77 I	50 P	82 I
Cadmium chloride	98 –	99 –	67 P	93 P
Cesium chloride	100 –	113 –	102 –	104 –
Mercuric chloride	84 L	93 –	8 P	75 L
Methyl Mercury	21 L	0 I	0 P	12 L
Nickel oxide	100 –	94 –	274 –	123 –
Selenium oxide	99 –	73 I	38 P	82 I
Vanadium pentoxide	100 –	97 –	52 P	92 P

Fitness of 100 indicates no effect, values less than 100 indicate toxicity. Effects are designated as L. = lethal, I = inhibitory, P = phenotoxic, – = no effect.

biphenyls from a point source. The sediments were extremely toxic to *P. redivivus*, with lethal effects and an overall fitness value of 10. Samples more remote from the point source also had significant toxic effects, but fitness increases as the distance from the point source of PCBs increased.

TABLE 2. The results of the *Panagrellus redivivus* bioassay on elutriates of sediments from a polychlorinated biphenyl (PCB) contaminated harbor.

Site	Effect	Fitness
1	Inhib.	73
2	Inhib.	64
3	Lethal	52
4	Lethal	48
5	Lethal	56
6	Lethal	42
7	Lethal	59
8	Inhib.	66
9	Inhib.	78
10	Inhib.	68
By outflow	Lethal	10

Last data point is by the outflow of the PCB source. Sites 1–9 sequentially represent points from one end of the harbor to the other. Sediments from sites 5 and 6 are most accessible to the outflow.

The test provides a cost-effective "yardstick" as to where the combined effects of toxicants is greatest.

The *P. redivivus* bioassay has been used to determine the relative toxic effects of fractions of sediments at several sites along river systems (20,25,26). Sediments were extracted, and eight fractions of increasing polarity were prepared. These fractions were ranked on the basis of observed toxic effect, with highest priority assigned to fractions causing significant toxic contamination of sediments occur and what broad class of compounds are primarily responsible for toxic contamination. Parallel with the *P. redivivus* assay, mutagenicity tests using *Salmonella typhimurium* (3,4,26) were performed on the fractions. The nematode bioassay was found to be at least as sensitive as the Ames test in detecting toxicity in these fractions. However, the nematode test costs less than 10% of the cost of the *Salmonella* bioassay.

The *P. redivivus* bioassay also was used to examine the partitioning of toxic contaminants between the water column, sus-

pended sediments, and bottom sediments (20). The suspended sediments at each site showed significant toxicity. In agricultural areas, the toxicity was greatest during spring runoff, whereas at sites associated with specific localized industries or municipalities, toxicity was greatest in the fall. The bottom sediments at some sites showed significant toxicity in the spring and negligible toxicity during the fall. The high toxicity in the spring probably results from the settling toxic suspended sediments over the winter. The level of toxicity in the water column is much lower than in the sediments.

The *P. redivivus* bioassay also has been used to locate the types and distribution of toxic materials that bioaccumulate in fish tissues (26,27). Red suckers (*Moxostoma macrolepidotum*), which are benthic suction feeders, and northern pike (*Esox lucius*), carnivorous feeders, were exposed to mercury during the 1960s and early 1970s in the Winnipeg River system. In pike, toxicity was found in brain tissue of large, older fish, but not in small, younger fish, reflecting the effectiveness of the cleanup measures initiated in the mid-1970s. Suckers had no toxicity associated with brain tissue but had toxic material in fatty tissue, which correlated with a spill of a nonpolar organic compound. Suckers upstream of the spill site did not have toxic material in their tissues, as determined by the *P. redivivus* bioassay (27).

The nematode bioassay is as sensitive as other widely used bioassay systems. In a study of the toxicity of a random sample of 117 oil well sump fluids, tests on a 50% dilution of the sump fluid produced significant toxicity with the *Panagrellus* bioassay in 97 samples. In dose-response studies, these sump fluids produced a significant EC50 using the Microtox test in 89 samples and significant lethality in 3-month-old rainbow trout during a 96-hour exposure in 75 samples. Thirty process waste waters were tested by the *P. redivivus* bioassay, by the 96-hour trout lethality assay, by the Microtox test, by the *Salmonella typhimurium* mutagenesis assay, and by two mutagenesis assays using *P. redivivus* (1,12). The *P. redivivus* bioassay found all samples to produce significant effects at a 10% dilution. The Microtox test detected 26 toxic samples, whereas the fish test found 22 toxic samples. The Ames test found four of the samples to be mutagenic, whereas the nematode mutagenesis assays detected mutagenesis in the same four samples and in four additional samples. These results demonstrate the utility of the nematode test as a cost-effective member of a suite of bioassays that can be used to evaluate the toxic potential or the efficacy of detoxification measures of complex contaminated sediments, sumps, and sludges.

Another application of the *Panagrellus* bioassay is in the evaluation of indoor air quality. One application has been in resolving the causality of urea-formaldehyde foam toxicity (29). In Canada an estimated 50,000 homes were insulated with urea-formaldehyde foam insulation (UFFI). In some of these homes residents complained of various health disorders, especially headache, fatigue, and nausea. The use of UFFI was subsequently banned in Canada because of the mutagenicity of formaldehyde. However, the issue of UFFI toxicity has not been resolved. Some researchers claim there are no adverse health effects of UFFI; others maintain that formaldehyde is the cause of the complaints, that a minor offgas of UFFI causes the toxicity, or that bacterial or fungal growth on UFFI causes the health complaints. The nematode bioassay was used to evaluate the biological effect of samples of UFFI removed from three Winnipeg homes. The UFFI samples were labelled as to the side of the homes from which they came, and were divided into east, west, north, and south samples, and tested as 1% filtrates of 10% slurries of phosphate buffer and foam. Of a total of 52 UFFI samples examined, 27% were toxic; 12 samples were inhibitory and 2 were phenotoxic. However, 25 of the samples (48%) were stimulatory. The east wall had the lowest frequency of toxic (inhibitory or phenotoxic) samples. To test if offgasses were the toxic constituent, foam samples were autoclaved at 100 C for 20 minutes and 15 psi. Of 28 autoclaved UFFI samples, only 3 (6%) showed toxicity; 2 were inhibitory, 1 was phenotoxic. Twenty-seven of the autoclaved foam samples (56%) were stimulatory. These results lead to two conclusions:

1. There is a toxic component in some UFFI samples. The presence of this component is probably the result of the history of the foam, and correlates with the sides of the home exposed to solar heating.
2. Many UFFI samples are stimulatory in the nematode bioassay, suggesting that they contain a metabolizable component that would promote the growth of bacteria and fungi.

A more direct method of evaluating indoor air quality involves the use of passive air samplers and the application of the *P. redivivus* test to the collected materials (30). Two samplers, one to passively collect organic vapors and another to collect airborne particles, have been developed. These samplers are sent to the sites to be tested, opened for a 7-day period at the tested site, and then resealed and returned for extraction and analysis by the nematode bioassay. The 7-day sampling period was selected to permit a sampling of the range of activities over a weekly cycle at the tested site. The absorbent sampler, containing activated molecular sieve 13X, is extracted with methanol, and the extract tested at 3% with the nematode bioassay. the particle sampler, containing sterile M9 buffer, is tested at a 10% dilution. The sampler–bioassay system provides a cost-effective method for the objective determination of indoor air quality.

The air sampler system has been tested in both workplaces and residences. A total of 724 absorbent samplers and 561 particle samplers have been tested in 32 different workplaces and 23 different residences. In workplaces, 19% of the tested sites had gaseous contaminants producing inhibitory effects and 14% of the sites had gaseous contaminants producing growth stimulation. Toxic particles were found at 24% of the tested workplace locations, with only 7% of the sites having stimulatory particles. In residential air, 21% of the absorbent samplers detected inhibitory gasses and 18% detected stimulatory gasses. The particle samplers placed in residences found 27% of the sites to have inhibitory particles and 5% to have stimulatory particles.

The detected effects correlate well with specific contaminants in air. For example, in a blind study with absorbent samplers placed in a restaurant, inhibitory effects were detected in the ducting of the main fume hood and in the smoking area of the dining room. In office buildings, inhibitory gasses often can be associated with areas where solvents are extensively used and with smoking areas. Stimulatory vapors are usually found in areas where workers complain of headache, eye irritation, and fatigue—symptoms of a mild allergic response.

Other nematodes have been used for ecotoxicological studies. The marine nematodes *Diplolaimelloides bruciei* and *Monhystera microphtalma* have been used for bioassays similar to *P. redivivus*. These species, which entrain their postembryonic development to their environmental conditions, show sensitivities to organic compounds and metals similar to those of *P. redivivus* (7,8). Lew et al. (18) developed a genetic test using reversion of mutants of *C. elegans*.

Conclusions

Laboratory studies using nematodes show promise in ecotoxicological studies. Of course, care must be exercised in extrapolating laboratory results to actual field conditions where other factors mitigate. Laboratory assays using nematodes can be used as yardstick upon which further testing or detoxification strategies can be based.

In the near future, ecotoxicological testing will involve the collaboration of field studies with lab studies to determine the relationship between contaminants and biological effects (28). With more than 65,000 new chemicals produced in the United States alone between 1945 and 1984 and only 2% satisfying legal requirements relative to toxicity criteria, there is a need for rapid, cost-effective bioassays to determine toxicity to the environment. The nematode bioassay provides one such system, producing quantitative data on several biological endpoints.

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